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REPORT NUMBER 812

ANTARCTIC ISOLATION AND ASSOCIATED CHANGES IN SALIVARY BACTERIA

by

Robert G. Esquire, CDR, DC, USN

Bureau of Medicine and Surgery, Navy Department
Research Work Unit MR041.20.02-6025.05

Released by:

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Commanding Officer
Naval Submarine Medical Research Laboratory

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SUMMARY PAGE

THE PROBLEM

Anticipated deployment into undersea, extraterrestrial and other uninhabitable areas requiring controlled-atmosphere shelters, suggests the need for broad understanding of human response to environmental extremes. The salivary oral flora of man not only may reflect overall biologic reaction, but also represent an obvious source of microbial transmission.

FINDINGS

Salivary acidogenic and bacterial fluctuations were observed in two groups of personnel during the Antarctic winter. Each group was exposed to different degrees of environmental stress. Salivary lactobacillus and presumptive Streptococcus salivarius counts were shown to be consistently different between the two study groups.

APPLICATIONS

The purpose of this study was to further refine earlier indications that human response to the extreme biotic stress of Antarctic survival was reflected in salivary bacterial population shifts. Long term study of the oral flora ecosystem may focus on interparametric biologic relationships. The potential for establishment of correlations with general physiologic conditions suggests an avenue for developing the use of saliva as an ancillary vehicle for monitoring health under deployed conditions where venipuncture for conventional blood chemistry is not feasible.

ADMINISTRATIVE INFORMATION

This investigation was conducted as part of Bureau of Medicine and Surgery Research Work Unit MR041.20.02-6025 - Study of Oral Health in the Antarctica. The present report is the 23rd and final report on this work unit. It was submitted for review on 29 May 1975, approved for publication on 5 June 1975 and designated as NavSubMedRschLab Report No. 812.

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ABSTRACT

Salivary studies were performed in an isolated community of fifty-one subjects during the Antarctic winter. Salivary acidogenesis, as measured by the Snyder Test, decreased significantly. Sustained differences in mean counts determined on media selective for lactobacilli and streptococci were observed as a function of relative levels of outdoor exposure. Differences in lactobacillus counts of indoor and outdoor workers paralleled findings reported in an earlier Antarctic study. Streptococcal growth on mitis salivarius agar had not been heretofore studied in Antarctica and S. salivarius counts varied inversely with lactobacillus counts. These findings appear to differentially relate to factors of oral health care, diet, environmental exposure and herd immunity.

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ANTARCTIC ISOLATION AND ASSOCIATED CHANGES IN SALIVARY BACTERIA

INTRODUCTION

The relationship of lactobacilli and streptococci to dental disease has long been a subject of extensive dental research effort. Direct relationships linking these particular organisms with acid production and caries abound in the literature.[1,2,3,4]

Although early Antarctic explorers indicated many dentally oriented problems,[5,6,7] recent investigators have reported a low incidence of oral disease.[8,9,10] Many reasons can be suggested as contributing to this phenomenon, probably the most significant being that preventive dental care has been considerably upgraded. Exposure to the harsh, relatively abiotic environment may also lower physiologic activity of oral bacteria related to oral disease.

During the Austral Winter of 1957, salivary pH levels and lactobacillus counts were shown to diminish with exposure to the Antarctic elements.[11,12] More recently, during the Austral Winter of 1966, the relationship of salivary acidogenic levels to environmental exposure complemented the 1957 pH findings.[13] These earlier studies suggested the need for expansion of effort to further define the dynamics of oral flora shifts during the Austral Winter. To assay the entire salivary flora would require logistic support of massive proportions. During the Austral Winter of 1971, however, it was possible to study a few representative bacterial groups. Since the streptococci and lactobacilli make up about half of the total oral flora of virtually all humans,[14] it seemed important to study these groups.

MATERIALS AND METHODS

Fifty-one volunteer subjects were selected from the 200-man wintering-over party at McMurdo Station, Antarctica, for salivary bacterial assay. The subjects were classified into two groups (outdoor or indoor) on the basis of degree of exposure to the Antarctic environment. Most outdoor workers were employed by the Public Works Department and consisted of personnel engaged in electrical line maintenance, fuel distribution and snow clearing. The indoor workers were primarily administrative, medical and supply personnel. Homogeneity of groups was supported by standard error of the mean comparisons of the following characteristics: Age, DMFT (Decayed, Missing, Filled Teeth) and DMFS (Decayed, Missing and Filled Tooth Surfaces) (Table 1).

Environmental Conditions and Sampling Technique.

The study commenced on the 39th day of the wintering-over party's 224-day isolation period. This covered a span of 185 days bracketing the Austral Winter of 1971. During this period, physical contact with the rest of the world ceased. Ships could not travel the frozen sea and airplane travel was severely restricted because hydraulic

Table 1 - Similarity characteristics of Antarctic indoor and outdoor workers.

		Mean	Variance	Standard deviation	Standard error	n
Age	Indoor workers	29.7	61.3	7.8	1.5	29
	Outdoor workers	26.1	42.8	6.5	1.4	22
Decayed, missing and filled teeth (DMFT)	Indoor workers	15.2	31.9	5.6	1.1	28
	Outdoor workers	16.1	39.4	6.3	1.4	22
Decayed, missing and filled surfaces (DMFS)	Indoor workers	31.0	216.6	14.7	2.9	28
	Outdoor workers	34.3	274.2	16.6	3.6	22

landing systems tended to freeze. Supplies of fresh fruit, vegetables, milk and eggs were exhausted within the first 60 days of isolation, not to be resupplied until a single flight was able to negotiate the trip from New Zealand on the 197th day of isolation. Climatic conditions included gradations of daylight and darkness ranging from minimal to total; low humidity ranging from 5 to 24%; and extreme cold ranging from -40° to +10°F. Indoor temperatures were maintained at about 70°F.

Single samples were obtained on each subject seven times during the study, at intervals of about 27 days. Approximately seven milliliters of paraffin-stimulated whole saliva was obtained in a sterile vial from each subject immediately upon arising. Each man was instructed to:

DONATE SALIVA IMMEDIATELY UPON ARISING FROM A PERIOD OF REST OR SLEEP

1. Do not smoke, drink, brush teeth or eat before sample is taken.

2. Place wax into mouth to warm to body temperature.

3. Then chew wax with all teeth by moving it around your mouth.

4. Expectorate saliva (not the wax) into vial as the saliva accumulates in your mouth.

5. Fill vial 3/4th full and replace cap securely.

6. Bring vial immediately to the Dental Staff.

Periodic expeditions forced occasional omissions in sampling. Corresponding adjustments were subsequently made in the number of subjects included in each analytical matrix, depending on the requirements of the statistical test applied.

Specimen Processing.

Specimens were inoculated to appropriate selective media, usually within an hour after collection and processing.[15] The collection vials were shaken in a Jay Shaker (Eberbach Corporation, Ann Arbor, Michigan) at 60 cycles per second for seven minutes. One milliliter transfers were immediately made to 9 ml and 99 ml dilution blanks of 0.2% Yeast Extract Broth (YEB), (DIFCO, Detroit, Michigan). Serial dilutions ranged from 10^{-1} to 10^{-8} depending upon which bacterial types were being assayed during a given study period. Since media supply limitations prevented use of all media at each of the seven sampling periods, specific assays were arranged to be performed at intervals most representative of the total isolation period: early, middle and late. Each dilution was hand shaken 30 times immediately prior to spreading 0.1 ml over the surface of the media using metal spreaders. All dilution assays were performed in duplicate for each type medium used. Snyder Test Agar prepared in 10 ml tubes was melted, and tempered to 45°C. Duplicate tubes were inoculated with 0.2 ml of whole saliva, and mixed by hand-rolling the molten suspension. All plates and tubes were incubated at 37°C.

Media and Organisms.

Commercially prepared media (DIFCO, Detroit, Michigan) were used throughout this study. Acidogenesis was measured in Snyder Test Agar by recording the degree of color change of the bromcresol green indicator over a 72-hour period. Lactobacillus counts were made on Rogosa Agar, enriched by adding 200 ml tomato juice filtrate to 800 ml of distilled water. Streptococcal counts were determined on Mitis-Salivarius Agar. All bacterial types were reported on the basis of presumptive identification derived primarily by colonial growth characteristics on selective/differential media with periodic confirmatory gram-staining.

Measurements.

Acidogenesis in Snyder Test Agar was assigned a value of zero to four depending on the degree of medium color change from green to yellow.

Individual lactobacillus levels varied over a wide span. Acceptable counting ranges were usually found at the 10^{-1} to 10^{-5} dilution levels with a few extensions to 10^{-7}. Lactobacillus counts were determined after four days of incubation.

Streptococci usually showed acceptable counting ranges at the 10^{-5} to 10^{-7} dilution levels. Presumptive counts for S. salivarius were determined at 48 hours of incubation based on the presence of large "gumdrop" colonies.[16,17] These plates were then reincubated for an additional four days, at which time presumptive counts for S. mitis[16,17] were determined based on the presence of minute blue colonies. These counts actually were probably a heterogenous mixture of oral streptococci and the designator "S. mitis" is used in this report primarily for morphologic colonial identity in describing differential counts on this selective media. Counts for both colonial types

were summated in order to provide total streptococci values for growth on Mitis-Salivarius Agar.

Statistical Treatment.

Duplicate plate counts on each subject were in close agreement. These were averaged and the resulting numbers entered as raw data in analytical matrices. The high degree of variability in human oral flora studies has historically frustrated attempts of investigators to quantitate salivary bacteria.[18,19] Counts obtained in this study proved no exception.

Parametric statistical analysis was complicated by high variability in bacterial populations between individuals. Variability within individuals was not as marked. Lactobacillus counts ranged from less than 100/ml to $12,700 \times 10^3$/ml saliva. Counts on Mitis-Salivarius media ranged from 6×10^6 to $2,378 \times 10^6$/ml saliva. Recurrence of extreme counts suggested that they were not products of artifaction and that exclusion of these subjects from the experimental regimen would be an unfair approach to characterizing the salivary flora of this population. Preliminary statistical analysis on individual raw count values produced variances too diverse for parametric comparisons of raw mean scores. Procedures for "normalizing" the data, similar to those used in Brown's[20] salivary bacterial studies with nonhuman primates, were attempted in order to more closely equalize variances to satisfy primary requisites[21] for the use of analysis of variance to compare means. Since raw score variances were roughly proportional to their means squared, the standard transformation of log (x+1) was selected to convert each average count for entry into the calculation matrices.[22] This had the desired effect of narrowing variance ranges and minimizing the impact of extreme count values, thus making feasible the inclusion of all data points on all subjects studied. Analyses of variance were performed and significant differences localized by the multiple-comparisons methods of Newman-Keuls and Duncan.[23] As a validity check, corresponding non-parametric chi square tests were performed. While the degree of parallelism in detection of significant differences was most encouraging, a few disparites still need to be resolved before the transformation method can be recommended as a suitable general procedure for parametrically handling salivary bacterial population data from randomly selected human subjects.

For purposes of this paper, probability-estimate (P) values for data obtained from plate counts are reported based on the non-parametric chi-square test with one degree of freedom. Differences between groups of indoor and outdoor workers are compared according to the median test. By the 39th day of isolation it was felt that both groups had been equally exposed to the same living conditions, with the exception of occupational activities which placed one group outdoors more frequently. Initial measurements obtained from the first sampling were considered early isolation period control values. Within-group comparisons against control values are reported according to the sign test.[22]

Snyder tests for acidogenesis, on the other hand, produced data with sufficient homogeneity of variance to permit the use of parametric statistics for comparison of means. One-way analysis of variance was used for between-group comparison of means of indoor and outdoor workers. Within-group comparisons of serial measurements were performed by two-way repeated measures analysis of variance and significant differences localized by the multiple-comparison method of Newman-Keuls.

RESULTS

Snyder Tests.

Mean measurements of acid production for indoor and outdoor groups decreased, becoming significantly lower than control at about 100 days into the isolation period. This decline was progressive and reached its lowest point late in the isolation period (175-194 days). The final testing period (202-221 days) showed a sudden increase in acid production to control level. There was no statistically demonstrable difference in acidogenesis between indoor and outdoor worker groups (Figure 1, Tables 2, 3, and 4).

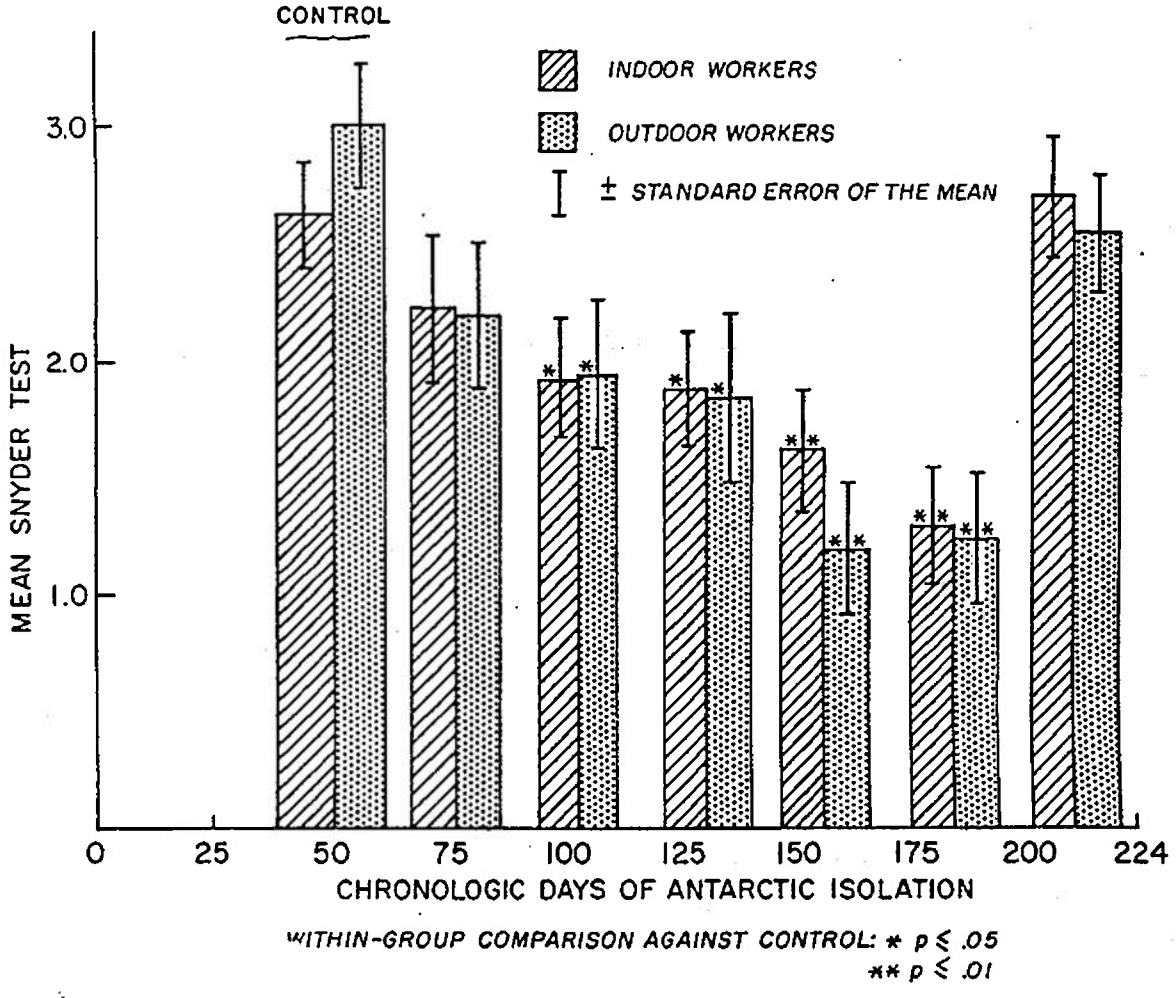

Fig. 1. Comparisons of whole saliva acidogenesis as measured by Snyder Tests on 47 subjects wintering-over in Antarctica. Indoor workers (n = 27) vs. Outdoor workers (n = 20)

Table 2 - Comparisons of Snyder tests. Indoor (n=27) and Outdoor (n=20) Workers

Antarctic isolation day number	Environmental exposure group	Mean ± SE	Q_w	f_b
39-62 (April control)	Indoor workers	2.63 0.23		1.100
	Outdoor workers	3.00 0.26		
68-87 (May)	Indoor workers	2.44 0.31	1.23	.009
	Outdoor workers	2.40 0.31	2.53	
95-112 (June	Indoor workers	1.93 0.23	4.61*	.004
	Outdoor workers	1.95 0.31	4.43*	
112-141 (July)	Indoor workers	1.89 0.24	4.80*	.009
	Outdoor workers	1.85 0.36	4.85*	
148-167 (Early August)	Indoor workers	1.63 0.26	6.49**	1.231
	Outdoor workers	1.20 0.28	7.59**	
175-194 (Late August)	Indoor workers	1.30 0.25	8.63**	0.016
	Outdoor workers	1.25 0.27	7.38**	
202-221 (September)	Indoor workers	2.70 0.25	0.45	0.164
	Outdoor workers	2.55 0.28	1.90	

Q_w = Neuman-Keuls Q value: Within-group comparison against April control

f_b = Analysis of Variance f value: Between-group comparison.

* = $p \leqslant .05$

** = $p \leqslant .01$

Lactobacillus counts.

At every measuring period, the mean lactobacillus count for outdoor workers was markedly lower than that for indoor workers. Comparisons of each group against joint median values revealed a statistically significant difference between indoor and outdoor workers moderately late in the isolation period (148-167 days). Mean values for the indoor worker group progressively decreased throughout the isolation period. Proportional decreases, as compared with control, reached a significant low during the final testing period (202-221 days). The outdoor worker group mean counts, conversely, showed a slight increase during the final sampling period (Figure 2, Table 5).

Table 3 - Snyder Tests. Within-group statistical analyses relative to duration of Antarctic isolation.

Analysis of variance for repeated measurements (two-way classification)

	Source of variance	Sum of squares	df	Mean square	f
Indoor workers (n=27)	Between sample periods	45.926	6	7.654	11.932**
	Within subjects (interaction term)	100.074	156	0.642	
Outdoor workers (n=20)	Between sample periods	53.686	6	8.948	
	Within subjects (interaction term)	128.314	114	1.126	7.950**

** = $p \leq .01$.

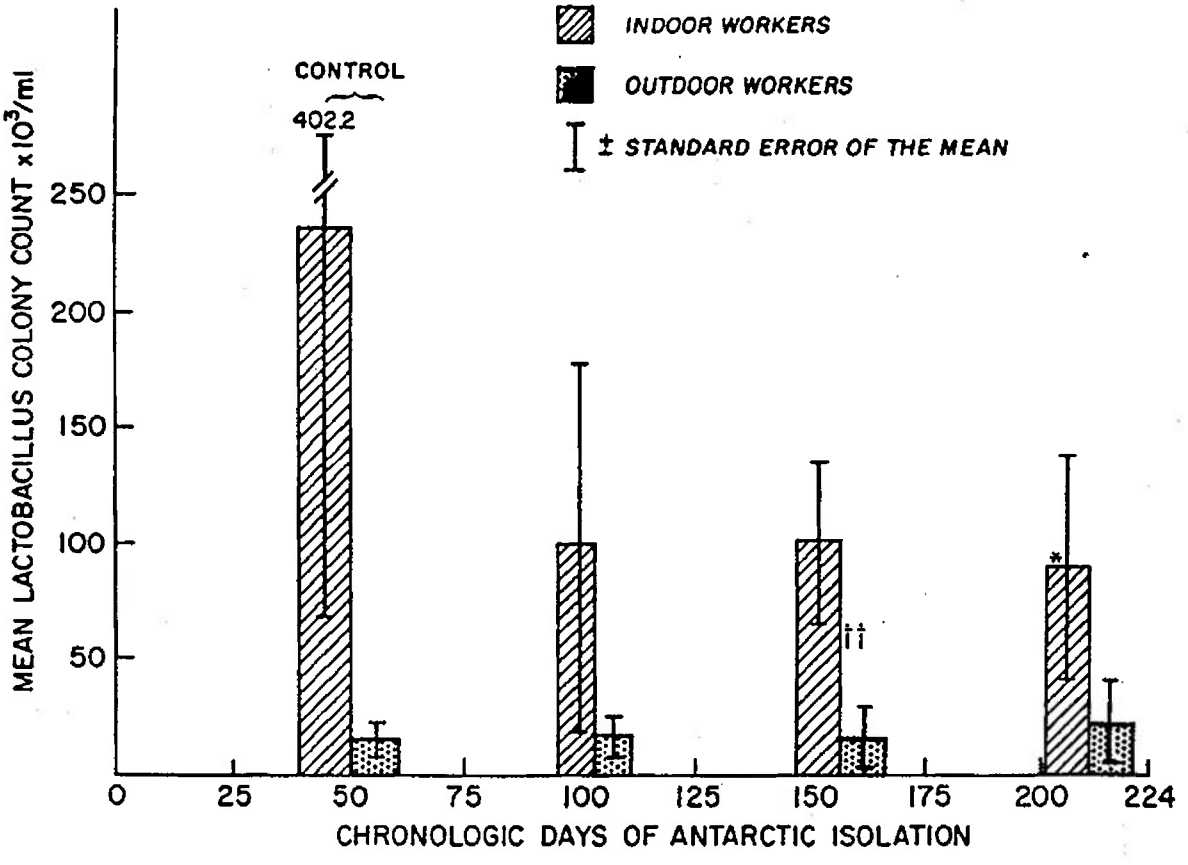

Fig. 2. Comparisons of whole saliva lactobacillus counts on Rogosa media for 51 subjects wintering-over in Antarctica. Indoor workers (n = 29) vs. Outdoor workers (n = 22)

Table 4 - Snyder Tests. Between-group statistical analyses relative to degree of Antarctic exposure.

Analysis of variance for independent groups (one-way classification).

Indoor (n=27) vs. outdoor (n=20) workers.

Antarctic isolation sampling period (day number)	Source of variance	Sum of squares	df	Mean square	f
39-62 (April)	Between groups	1.576	1	1.576	1.103
	Within groups	64.296	45	1.429	
68-87 (May)	Between groups	0.023	1	0.023	0.010
	Within groups	103.467	45	2.299	
95-112 (June)	Between groups	0.007	1	0.007	0.004
	Within groups	74.802	45	1.662	
112-141 (July)	Between groups	0.018	1	0.018	0.009
	Within groups	89.217	45	1.983	
148-167 (Early August)	Between groups	2.121	1	2.121	1.231
	Within groups	74.496	45	1.722	
175-194 (Late August)	Between groups	0.025	1	0.025	0.016
	Within groups	71.380	45	1.586	
202-221 (September)	Between groups	0.271	1	0.271	0.164
	Within groups	74.580	45	1.657	

Presumptive Streptococcal Counts.

Total Count

Plate-count values for both groups showed a cyclical trend. Control values started out low, increased significantly early in the isolation period (68-87 days), returned to control level midway through the isolation period (122-141 days), and then increased again late in the isolation period. During one of the late sampling periods (175-194 days), the indoor worker counts again showed a significant increase over control. Significant differences were not demonstrable between the two worker groups at any point during the study. (Figure 3, Table 6).

Table 5 - Comparisons of salivary lactobacillus counts (colony count x 10^6/ml)

Antarctic isolation sampling period (day number)	Environmental exposure group	Mean	±	SE	χ^2_w	N	χ^2_b	n
39-62 (April control)	Indoor workers	235.5		166.7			.74	29
	Outdoor workers	16.9		8.7				21
95-112 (June)	Indoor workers	98.4		79.5	2.45	20	1.19	29
	Outdoor workers	17.6		8.9	0.84	19		22
148-167 (August)	Indoor workers	100.8		35.5	.04	24	7.32††	29
	Outdoor workers	17.0		15.8	.21	19		22
202-221 (September)	Indoor workers	92.1		48.7	4.76*	21	0.56	29
	Outdoor workers	23.6		17.9	1.07	15		22

χ^2_w = Chi-square within-group comparison against April control (sign test)

* = $p \leq .05$ N = paired observations

χ^2_b = Chi-square between-group comparison (median test)

†† = $p \leq .01$ n = group size

Differential Counts

S. salivarius.

The mean presumptive S. salivarius count for the outdoor worker group was consistently higher than that for the indoor worker group. Between-group comparisons against the joint median showed the outdoor worker counts to be significantly higher during the early sampling periods (39-87 days). The indoor worker counts showed significant increase over their control counts both during this early sampling period and in the final sampling period (202-221 days). (Figure 4, Table 7).

S. mitis.

Presumptive S. mitis values for both groups demonstrated a pattern parallel to that described for total colony counts on this media. Group comparison against the joint median demonstrated outdoor worker counts to be significantly higher than

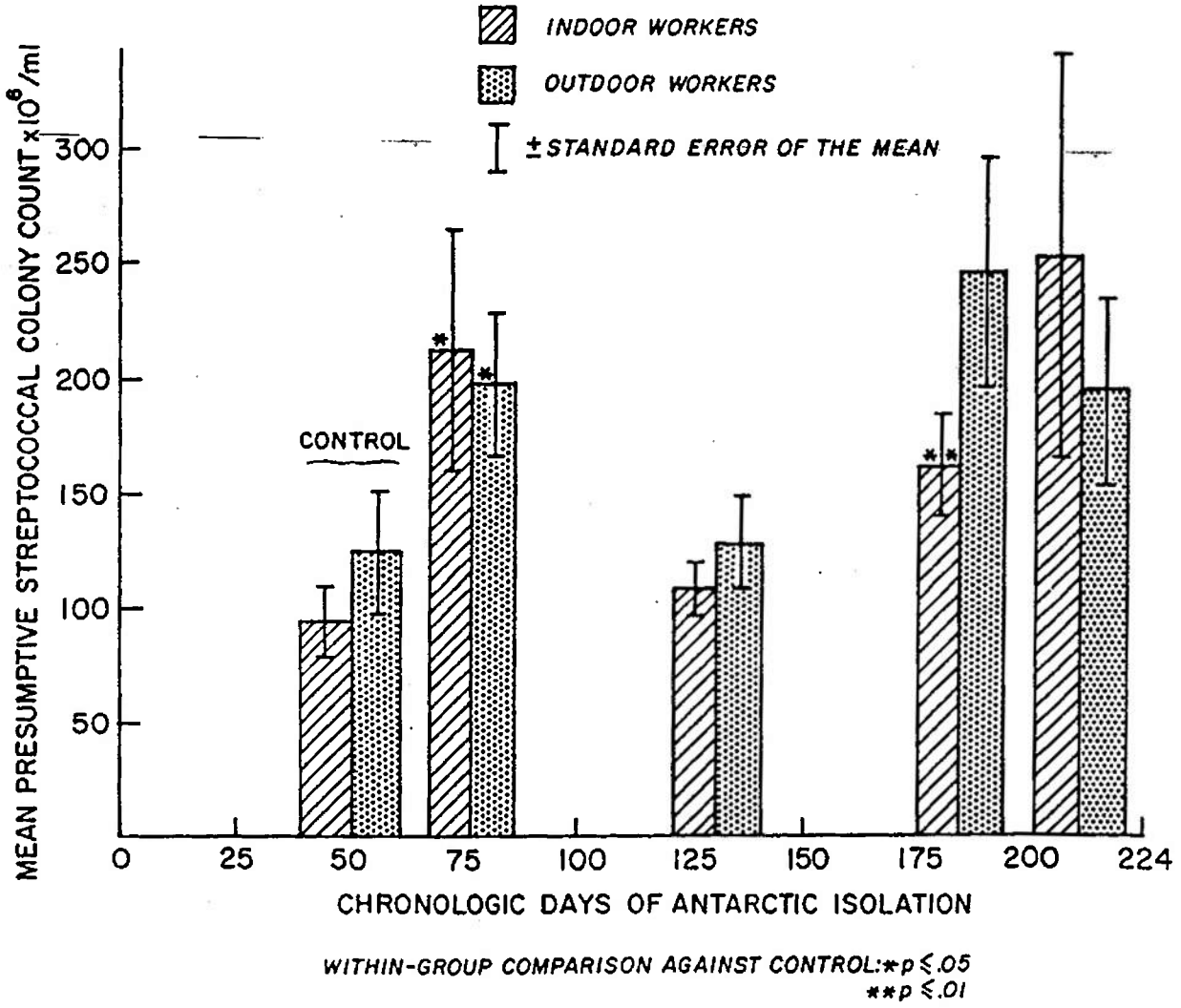

Fig. 3. Comparisons of total presumptive streptococci counts on Mitis-salivarius media for 51 subjects wintering-over in Antarctica. Indoor workers (n = 29) vs. Outdoor workers (n = 22)

those for the indoor group during one of the late sampling periods (175-194 days). The indoor workers showed significant increases over control during both this period and the early sampling period (68-87 days). (Figure 5, Table 8).

Table 6 - Comparisons of presumptive salivary streptococcal total counts on Mitis-Salivarius media (colony count x 10^6/ml)

Antarctic isolation sampling period (day number)	Environmental exposure group	Mean	± SE	χ^2_w	N	χ^2_b	n
39-62 (April control)	Indoor workers	95.3	15.0			2.12	27
	Outdoor workers	126.3	27.9				21
68-87 (May)	Indoor workers	213.4	51.7	4.82*	28	0.78	29
	Outdoor workers	197.6	31.6	4.76*	21		21
122-141 (July)	Indoor workers	109.2	12.2	0.04	28	0.32	28
	Outdoor workers	128.2	20.2	0.76	21		22
175-194 (Late August)	Indoor workers	163.0	23.3	10.32**	28	3.30	29
	Outdoor workers	246.4	50.9	1.71	21		22
202-221 (September)	Indoor workers	253.1	89.3	1.75	28	0.74	29
	Outdoor workers	195.4	40.6	0.19	21		21

χ^2_w = Chi-square within-group comparison against April control (sign test)

* = $p \leqslant .05$ N = paired observations

** = $p \leqslant .01$

χ^2_b = Chi-square between-group comparison (median test)

n = group size

DISCUSSION

Living conditions at McMurdo Station Antarctica, have improved markedly since the early salivary pH studies[11] revealed differences related to degree of environmental exposure. Because of warmer, more comfortable quarters; protected, heated cabs for outdoor heavy equipment and billeting of virtually all personnel in the same building, it was expected that differences between indoor and outdoor workers would be undetectable. Since specimens were collected upon arising, re-establishment of individual normal salivary bacterial densities would have been expected to occur during sleep. Consistent with these expectations, results of Snyder Tests in this study demonstrated no significant differences in acidogenic levels between indoor and outdoor workers. This relationship was at variance with findings of Kasenchak[13] in 1966, when living conditions were far more spartan. The overall profile observed in this study however, demonstrates a significant,

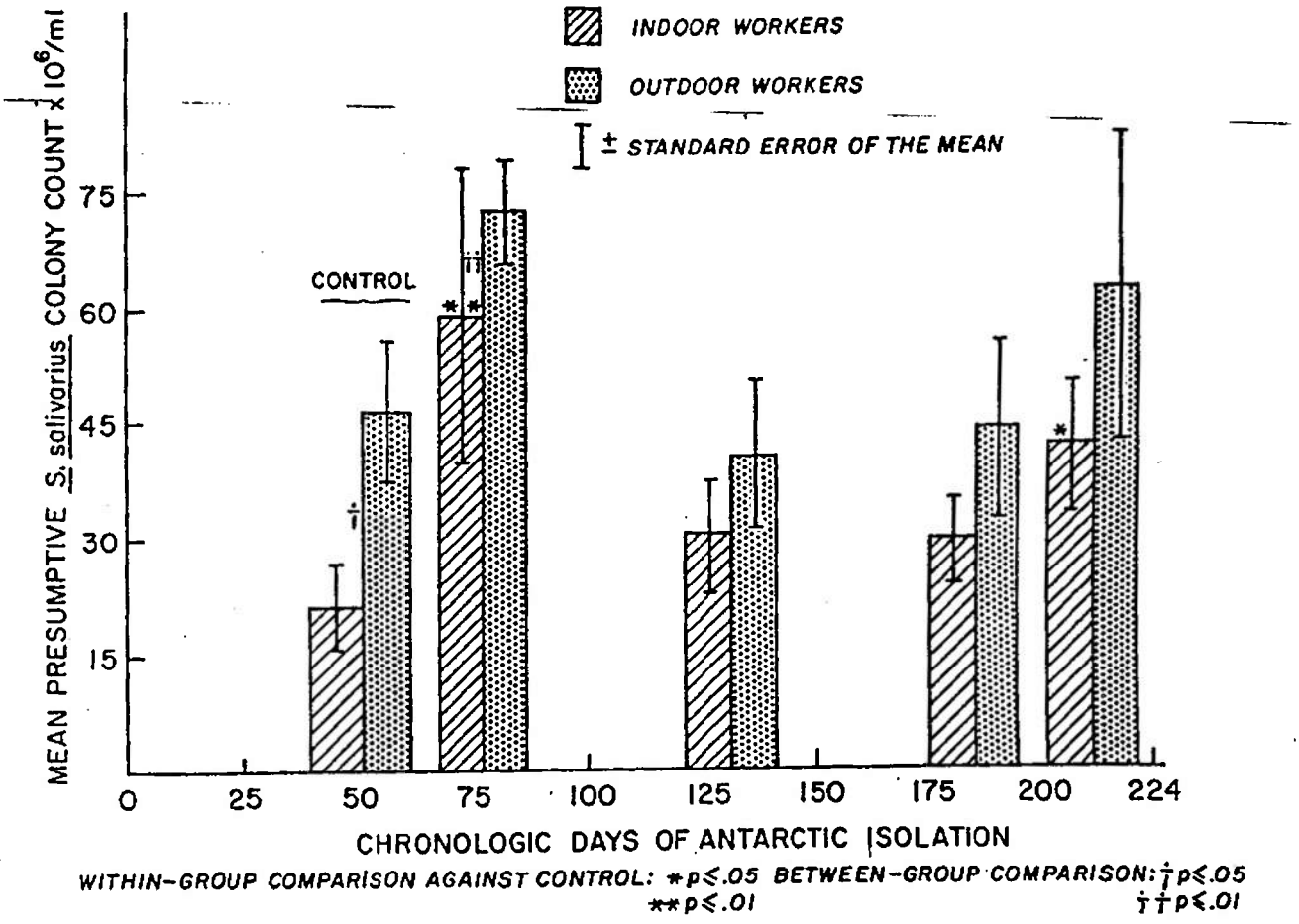

Fig. 4. Comparisons of differential presumptive S. salivarius counts on Mitis-salivarius media for 51 subjects wintering-over in Antartica. Indoor workers (n = 29) vs. Outdoor workers (n = 22)

progressive reduction in acidogenesis throughout the isolation period, except for a sudden return to control level during the final sampling period just prior to re-opening of the Station. Several factors may have produced this effect:

1. Arrival of a single flight brought in fresh food and new personnel. Fresh dairy products, fruit and vegetables (which had not been available since approximately the 60th day of the isolation period) arrived on the 197th day of isolation, just prior to commencement of the final sampling period. This flight also brought in approximately 15 new personnel, 10 of whom stayed to assist the Wintering-over party's preparation for Station opening which would occur one month later. A few of these new personnel had "colds". Shortly after arrival of this flight, minor upper-respiratory illness was generally observed throughout the Wintering-over party.

2. Motivation for oral hygiene was periodically reinforced by the Dental

Table 7 - Comparisons of presumptive salivary S. Salivarius differential counts (colony count x 10^6/ml)

Antarctic isolation sampling period (day number)	Environmental exposure group	Mean	±	SE	χ^2_w	N	χ^2_b	n
39-62 (April control)	Indoor workers	21.4		5.7			5.48†	28
	Outdoor workers	46.9		9.1				20
68-87 (May)	Indoor workers	59.2		20.1	7.84**	25	6.65††	29
	Outdoor workers	73.3		13.0	1.71	21		21
122-141 (July)	Indoor workers	30.8		7.5	0.15	27	0.07	29
	Outdoor workers	40.1		9.5	0.05	21		22
175-194 (Late August)	Indoor workers	30.0		5.8	1.75	28	0.87	29
	Outdoor workers	44.5		11.5	0.19	21		22
202-221 (September)	Indoor workers	41.2		8.6	4.48*	27	0.20	29
	Outdoor workers	62.9		20.1	0.19	21		22

χ^2_w = Chi-square within-group comparison against April control (sign test)

* = $p \leq .05$ N = paired observations

** = $p \leq .01$

χ^2_b = Chi-square between group comparison (median test)

† = $p \leq .05$ n = group size

†† = $p \leq .01$

Department throughout the isolation period. This pattern of acidogenic decline and return to control parallels the patterns of reduction and return to control of oral disease indices observed in these subjects (unpublished data) and in other Antarctic studies.[9,10,24] Since the final sampling was conducted during a period of intensive activity in preparation for station re-opening, improved behavioral patterns in oral health maintenance probably fell prey to occupational stress factors and plaque was permitted to accumulate.

The observation of markedly lower lactobacillus counts in the outdoor workers, however, tends to verify earlier indications of an environmental effect[12] on this bacterial group. The low levels of lactobacilli observed early in the isolation period in the outdoor group and late in the isolation period for both groups is in direct contrast to the high levels of acidogenesis demonstrated in their Snyder Tests. Snyder Test acidity has often been directly associated with density of lactobacilli.[25]

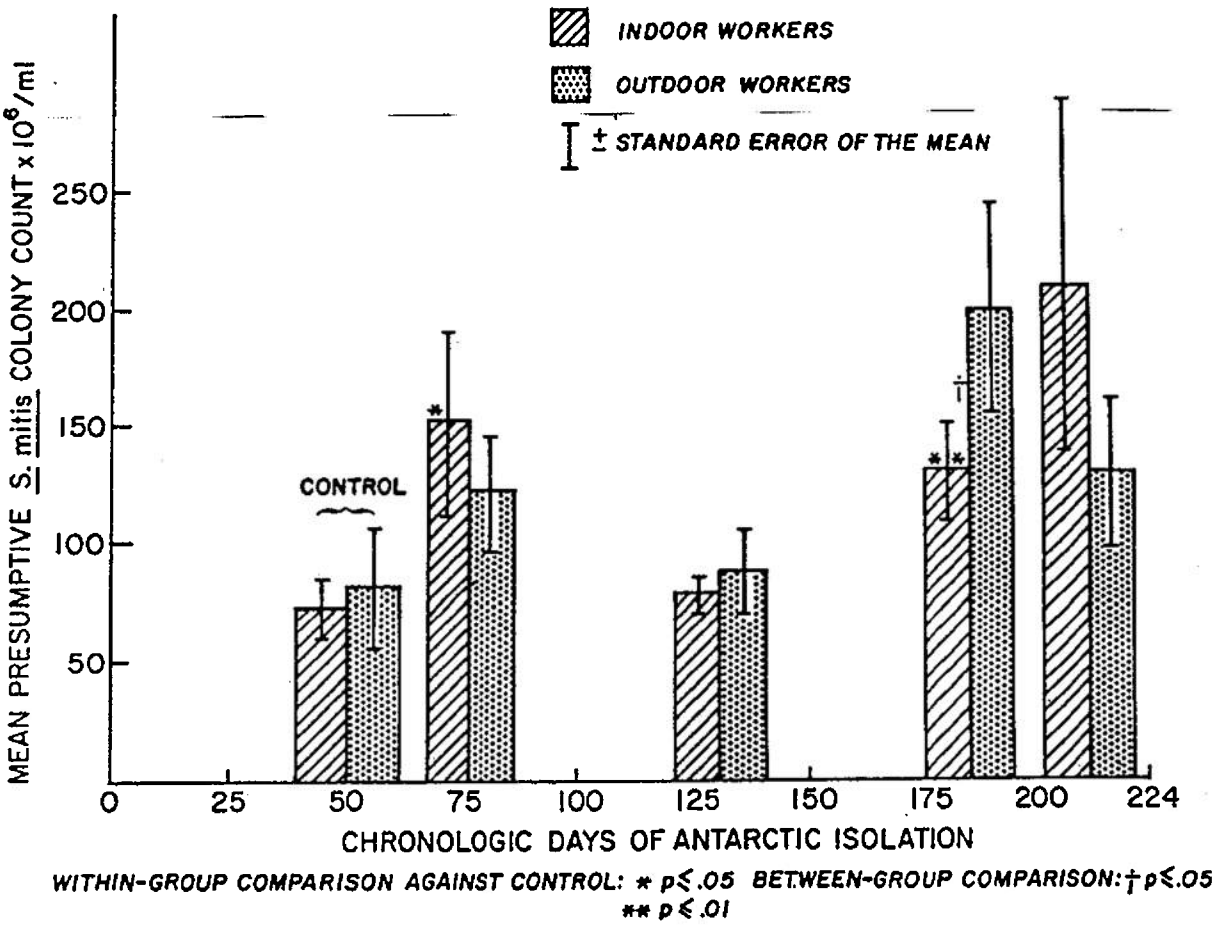

Fig. 5. Comparisons of differential presumptive S. mitis counts on Mitis-salivarius media for 51 subjects wintering-over in Antarctica. Indoor workers (n = 29) vs. Outdoor workers (n = 22)

Counts for presumptive streptococcal colonies, while at variance with high acidogenesis early in the isolation period, roughly seemed to parallel the Snyder Test profile showing reductions to a low point at the middle of the isolation period and progressive increase toward the end of Wintering-over. It should be noted, however, that commencement of the late increase in streptococcal levels preceded arrival of the first flight on the 197th day and that indoor and outdoor workers demonstrated statistically significant differences in both lactobacillus and S. mitis counts during a period of increased exposure on the part of the outdoor workers in airstrip preparation for the first flight (148-194 days).

An additional suggestion of environmental effect is the observation that differential counts for presumptive S. salivarius colonies were consistently higher in the outdoor workers, a difference which was statistically significant early in the isolation period

Table 8 - Comparisons of presumptive salivary S. mitis differential counts (colony count x 10^6/ml)

Antarctic isolation sampling period (day number)	Environmental exposure group	Mean	±	SE	χ^2_w	N	χ^2_b	n
39-62 (April control)	Indoor workers	73.9		12.0			1.15	28
	Outdoor workers	82.6		24.8				21
68-87 (May)	Indoor workers	154.1		38.6	4.32*	28	0.93	29
	Outdoor workers	124.3		25.7	1.71	21		21
122-141 (July)	Indoor workers	78.0		8.9	0.32	28	1.57	29
	Outdoor workers	88.1		17.9	0	21		22
175-194 (Late August)	Indoor workers	133.0		20.3	8.03**	28	4.02††	29
	Outdoor workers	201.9		44.9	1.71	21		21
202-221 (September)	Indoor workers	212.0		84.6	1.75	28	0.20	29
	Outdoor workers	132.6		32.5	1.71	21		22

χ^2_w = Chi-square within-group comparison against April control (sign test)

* = $p \leq .05$ N = paired observations

** = $p \leq .01$

χ^2_b = Chi-square between group comparison (median test)

†† = $p \leq .05$ n = group size

(39-87 days). While outdoor activity was not as extensive during this time (as compared with the late periods) adaptation to isolation, darkness and decreasing temperature may have still been occurring and could have had an effect on the flora of those workers who were more frequently exposed to the adverse elements.

The recurring observation that outdoor workers consistently demonstrated low lactobacillus and high S. salivarius counts as contrasted with the recurrent opposite bacterial relationship in the indoor workers suggests an ecological shift. It is hypothesized that the susceptibility of lactobacilli to the hostile environment permitted selective enrichment and hence growth of more resistant streptococci. Similar streptococcal-lactobacillus shifts reported by other investigators have been associated with full-mouth extractions[26] and oral radiotherapy.[27]

Presumptive S. mitis counts were much higher than those for S. salivarius, demonstrating their ability to mask the observed differences between

indoor and outdoor group S. salivarius counts when both counts were pooled. The overall profile for S. mitis directly paralleled that for total counts on this media. The pooling of counts also prevented detection of significance between indoor and outdoor worker S. mitis counts during one of the late sampling periods (175-194 days).

CONCLUSION

Individual diversity of salivary bacteria, while making assay a complex procedure, may provide a wide variety of indices to correlate with different factors relative to the general physiologic condition. In this study, acidogenesis appeared more related to local factors in the oral physiology concerning oral health maintenance and dietary change. Assay of two specific salivary bacterial groups, on the other hand, identified changes more suggestive of response to environmental stress. The observation that lactobacillus and streptococcal counts did not directly parallel the pattern of acidogenesis indicates other factors to be at work in decreasing salivary acidogenic potential. Salivary yeast and staphylococcal levels could very well play a significant role in this phenomenon.

Since the Antarctic atmosphere is relatively gnotobiotic, factors of herd immunity cannot be discounted. Sladen[28] found that nasal and pharyngeal carrier rates of Staph. aureus and Strep. pyogenes were sharply reduced or eliminated in men during a 12-month period of Antarctic isolation. Muchmore[29] hypothesizes that the relative absence of infections in Wintering-over personnel reflects a decrease in the number and varieties of microbial agents to which these isolated personnel are exposed. His report of neutropenia in healthy personnel Wintering-over at the South Polar Plateau shows a reduction in white blood cell count with a return to baseline. This pattern is curiously parallel to the salivary acidogenesis profile reported in this study.

The statistically significant salivary alterations in oral flora parameters observed in this study suggest an additional avenue for characterizing man's response to changes in his environment. The diagnostic value of whole saliva as a vehicle for assessing the physiologic state can probably be better defined in studies which simultaneously provide a broad spectrum of biometric analyses.

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